PATENT
U.S. Appl. Ser. No.; **09/271,02**
Attorney Docket No. **CONLINCO-03681**

APPENDIX 2

ISSN 0931-5985
Volume 101 (8) 271–312

Fett

Zeitschrift für Wissenschaft und Technologie der Fette, Öle und Wachse

Lipid

Journal for Science and Technology of Fats, Oils and Waxes

Inhalt / Contents

Forschungsbeiträge/Research Papers

Variations in isomer distribution in commercially available conjugated linoleic acid*

Martin P. Yurawecz[1], Najibullah Sehat[1], Magdi M. Mossoba[1], John A.G. Roach[1], John K. G. Kramer[2], and Youh Ku[1]

Conjugated linoleic acid (CLA) has been reported to have anticarcinogenic and antiatherogenic properties, to repartition body fat, to build bone mass, to normalize glucose tolerance, and to reduce hyperglycemia and diabetes. CLA products are now commercially available, and there is considerable interest in studying CLA because of this range of reported beneficial effects. However, little is known about the composition of these preparations. Representative commercial CLA products in capsule or liquid (aqueous or oily) form were analyzed for their CLA content and isomer composition using gas chromatography (GC), silver ion-high performance liquid chromatography (Ag$^+$-HPLC) and spectroscopic techniques. The content of CLA in the preparations varied widely. Based on the GC-internal standard technique, total CLA varied from 20 to 89% by total weight and 28 to 94% of total fat. One product contained no CLA. The isomer distributions were generally of two types: those with two major CLA positional isomers, and those with four major CLA positional isomers. All the CLA preparations in capsule form contained the four isomer mixture, while the liquid preparations contained from two to four CLA positional isomers.

Unterschiede in der Isomerenverteilung kommerziell erhältlicher konjugierter Linolsäure. Von konjugierter Linolsäure (CLA) wurde berichtet, daß sie anticanzerogene und antiatherogene Eigenschaften hat, Körperfett repartioniert, Knochenmasse aufbaut, Glukosetoleranz normalisiert und Hyperglykämie und Diabetes reduziert. CLA-Produkte sind jetzt kommerziell erhältlich, und es gibt wegen der oben aufgeführten positiven Effekte ein beträchtliches Interesse daran, CLA zu studieren. Allerdings ist wenig bekannt über die Zusammensetzung dieser Herstellungen. Repräsentative kommerzielle CLA-Produkte in Kapsel- oder flüssiger Form (auf Wasser- oder Ölbasis) wurden mit Hilfe eines Gaschromatographen (GC), Silberionen-Hochdruckflüssigkeitschromatographie (Ag$^+$-HPLC) und spektroskopischer Techniken auf ihren CLA-Inhalt und ihre isomere Zusammensetzung analysiert. Der Inhalt der CLA in den Herstellungen variierte stark. Auf der Basis der GC-internen Standardtechnik schwankten die gesamten CLA zwischen 20 und 89% bezogen auf das Gesamtgewicht und zwischen 28 und 94% bezogen auf den Gesamtfettanteil. Ein Produkt enthielt keine CLA. Die Isomerverteilungen untergliederten sich allgemein in zwei Typen: solche mit zwei Positionsisomeren und solche mit vier Positionsisomeren. Alle CLA-Herstellungen in Kapselform beinhalteten das Gemisch der vier Isomere, während die flüssigen Herstellungen zwischen zwei und vier der Positionsisomere enthielten.

1 Introduction

Conjugated linoleic acid (CLA) has been reported to provide direct [1] or indirect [2] protection against several types of cancer, atherosclerosis [3, 4], and diabetes [5]. CLA has also been reported to improve feed efficiency [6] and increase muscle [7, 8], and bone mass [9]. These results were generally obtained in experimental animals fed commercial CLA preparations containing approximately equal amounts of four *cis/trans* conjugated octadecadienoic (18:2) acids: 8 *trans*, 10 *cis*-18:2; 9 *cis*, 11 *trans*-18:2; 10 *trans*, 12 *cis*-18:2; 11 *cis*, 13 *trans*-18:2; and minor amounts of the corresponding *cis,cis* and *trans,trans* CLA isomers [10]. Thus, the contribution(s) of the specific isomers to the observed effects are not known. In contrast, natural products, such as milk, cheese, and meat from ruminant animals contain mainly rumenic acid (9 *cis*, 11 *trans*-18:2) [11–13] with minor amounts of 7 *trans*, 9 *cis*-18:2 [14] and other isomers [15–17]. The total CLA content in these natural products ranges from trace to 2% of total fatty acids [12, 18, 19].

The present study was undertaken to determine the content and distribution of CLA isomers in commercially available CLA capsules and liquid products with labels stating to contain CLA. The CLA isomers were analyzed by gas chromatography (GC) and silver ion-high performance liquid chromatography (Ag$^+$-HPLC) as their fatty acid methyl esters (FAME), and identified by GC-electron ionization mass spectroscopy (GC-EIMS) and GC-direct deposition-Fourier transform infrared (GC-DD-FTIR) spectroscopy as their 4,4-dimethyloxazoline derivatives [10, 14, 17, 20].

2 Materials and Methods

2.1 Chemicals

Representative CLA preparations were purchased locally, from specialty chemical companies, or from the World Wide Web. Several pure CLA isomers were obtained as their free fatty acids from *Matreya*, Inc. (Pleasant Gap, PA). Acetonitrile and hexane were UV grade. Other solvents were distilled-in-glass quality. 2-Amino-2-methyl-1-propan 1 (95%) was purchased from *Aldrich Chemical Company*, Inc. (Milwaukee, WI). A 10% solution of trimethylsilyldiazomethan in hexane was obtained from *TCI America* (Portland, OR).

[1] US Food and Drug Administration, Center for Food Safety and Applied Nutrition, USA.

[2] Southern Crop Protection, Food Research Center, Agriculture and Agri-Food Canada, Canada.

* Presented in part at the 52nd International Deutsche Gesellschaft für Fettwissenschaft (DGF) Congress in Magdeburg, Germany, Sept. 13–15, 1998.

Anhydrous $NaOCH_3$/methanol was purchased from *Supelco*, Inc. (Bellefonte, PA.).

2.2 Lipid extraction

A known weight (approximately 25 mg) of each product was dissolved in 2 ml 1 N KOH in ethanol (95%) and hydrolyzed overnight in the dark at room temperature. For quantitative analyses, one mg of eicosanoic acid (23:0) was added as an internal standard. After hydrolysis, 5 ml of H_2O and one ml of 6 N HCl were added and the free fatty acids were extracted three times with 5 ml diethyl ether/petroleum ether (1:1). The combined extracts were washed with H_2O and dried over anhydrous Na_2SO_4, and the solvents removed under a stream of argon. Aqueous CLA samples were first extracted with petroleum ether/diethyl ether (1:1), and 25 mg of the extracted lipids were treated as described above.

2.3 Derivatizations

FAMEs were prepared for GC by dissolving the free fatty acids in one ml of benzene/methanol (4:1) to which 0.5 ml of a 10% solution of trimethylsilyldiazomethane in hexane were added [21]. The reaction was allowed to stand for 0.5 h with occasional gentle shaking. Thereafter, five drops of glacial acetic acid were added with gentle shaking. The same amount of glacial acetic acid was added to each of the solutions to destroy excess yellow trimethylsilyldiazomethane. S me solutions did not become clear on addition of glacial acetic acid. Then 5 ml of H_2O were added, and the reaction mixture was extracted with one ml of isooctane. The extract was subsequently dried over anhydrous Na_2SO_4.

The 4,4-dimethyloxazoline (DMOX) derivatives were prepared to determine the double bond position of CLA isomers. Ten to 20 mg of the free fatty acid product prepared above was added to a screw cap reaction tube (1 ml) and a threefold excess (w/w) of 2-amino-2-methyl-1-propanol was added. The tube was purged with argon, capped, and heated at 170 °C for 0.5 h in an oven. DMOX derivatives were then partitioned into petroleum ether as described previously [22].

2.4 Gas chromatography

The analyses of the FAMEs were carried out using a *Hewlett-Packard* (Palo Alto, CA) model 5890 gas chromatograph fitted with a flame-ionization detector. A CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness; *Chrompack*, Bridgewater, NJ) was used, and H_2 was the carrier gas at a split ratio of 50:1. The column was operated at 75 °C for 2 min, then temperature-programmed at 5 °C/min to 185 °C, held for 30 min, followed by a second temperature program at 4 °C/min to 225 °C and held there for 33 min.

2.5 Ag^+-HPLC

The HPLC (*Waters 510* solvent delivery system; *Waters Associates*, Milford, MA) was equipped with an autosampler and 200-µl injection loops (*Waters 717*), a UV detector operated at 233 nm (*Waters 486* tunable absorbance), and a data system (*Waters Millennium™* version 2.15). A *ChromSpher 5 Lipids* analytical silver impregnated column (4.6 mm i.d. × 250 mm stainless steel; 5 µm particle size; *Chrompack*, Bridgewater, NJ) was operated at room temperature. The mobile phase was 0.1% acetonitrile in hexane and operated isocratically at a flow rate of 1.0 ml/min. The retention times varied slightly between runs due to the insolubility of acetonitrile in hexane. However, these changes did not affect the relative elution sequence of CLA isomers. Typical injection volumes were 5–15 µl at a concentration of 1 mg total FAME per ml.

2.6 Gas chromatography – electron ionization mass spectrometry

The GC-EIMS analyses were performed by using a *Hewlett-Packard* (model 5890, series II) GC coupled to a mass spectrometer (*Autospec Q* mass spectrometer) and a data system (*OPUS 4000; Micromass*, Manchester, UK). The GC-EIMS system utilized version 2.1 BX software. This system was used with a 50 or 100 m CP-Sil 88 fused-silica capillary column. The GC-EIMS conditions were: splitless injection with helium or hydrogen as the carrier gas and sweep was restored 1 min after injection. The injector and transfer lines temperatures were 220 °C. The column was operated at 75 °C for one min after injection, then temperature-programmed 20 °C/min to 185 °C, held there for 15 min, then temperature-programmed 4 °C/min to 220 °C, and held there for 45 min.

Tab. 1. Conjugated linoleic acid (CLA) methyl ester isomers, as % of total CLA, in 13 commercial CLA preparations as determined by silver ion-high performance liquid chromatography (Ag^+-HPLC).

Product	*trans,trans*				*cis/trans*[a]				*cis,cis*			
	11,13	10,12	9,11	8,10	11,13	10,12	9,11	8,10	11,13	10,12	9,11	8,10
1 aqueous	0[b]	0	0	0	0	0	0	0	0	0	0	0
2 oil	0	1.1	1.0	tr[c]	0	47.1	50.8	tr	0	tr	tr	0
3 oil	0	0.5	0.5	0	1.1	50.2	47.6	tr	0	tr	tr	0
4 oil	0	1.1	1.3	0	0	45.8	50.7	tr	0	1.1	0.1	tr
5 oil	0	0.6	0.6	0	0	54.0	43.5	0	0	0.7	0.6	0
6 oil	1.5	5.4	11.9	7.1	2.2	38.3	21.7	tr	0	2.3	6.2	3.5
7 capsule	0.7	2.7	2.8	0.5	19.0	32.1	25.6	15.6	0.7	0.4	tr	tr
8 capsule	0.8	2.7	2.5	0.4	16.8	33.9	27.1	14.2	tr	1.7	tr	tr
9 capsule	1.0	3.1	2.8	0.5	16.9	33.7	26.9	14.0	0.6	0.5	tr	tr
10 capsule	0.7	2.5	2.5	0.6	15.5	31.0	27.7	14.2	0.8	2.2	1.8	0.6
11 capsule	0.4	2.8	2.9	0.5	14.4	29.1	30.0	15.9	0.5	1.5	2.1	tr
12 capsule	1.3	3.3	3.4	1.1	19.8	25.9	21.6	16.5	1.2	2.7	2.4	1.0
13 oil	4.0	5.4	5.4	1.7	19.7	26.8	25.6	10.5	0.2	0.1	0.6	0.1

[a] The CLA isomers exist either in the *cis,trans* or *trans,cis* configuration. [b] 0, not detectable. [c] tr, trace (< 0.05%).

2.7 GC-direct deposition Fourier transform infrared spectroscopy

GC-DD-FTIR was performed using a *Bio-Rad* (Cambridge, MA) *Tracer™ GC-FTIR 60A* spectrometer system. This system was used with a 50 m CP-Sil-88 fused-silica capillary column as described previously [23,24].

3 Results and Discussion

Preparations of CLA were capsules or liquids that were water-based or oil-based; some contained non-lipid material. Their chemical compositions were not known. Based on the assumption that the CLA products consisted of esters, free fatty acids or combinations thereof, all products were hydrolyzed under alkali conditions and subsequently methylated by using trimethylsilyldiazomethane as catalyst to ensure preservation of the original distribution of CLA isomers [25].

Total fatty acid compositions of the 13 CLA products were determined. The internal standard (23:0) added to the CLA products provided a mean to determine th amount of total fatty acids in the original CLA mixture. When this total fatty acid value was compared to the 25 mg of starting material used, an approximate estimate of the non-lipids in the sample was obtained. The approximate amount of non-lipid material calculated for these products ranged from 3 to 28%. In addition, unidentified FAMEs ranged from 1 to 29%. The

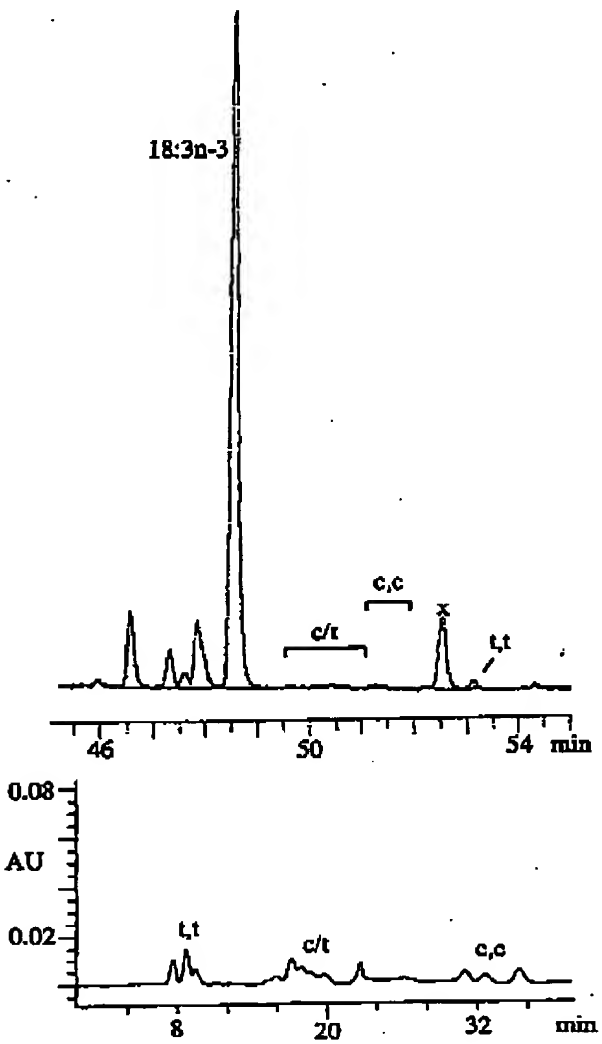

Fig. 1. Gas (upper graph) and silver ion-high performance liquid (lower graph) chromatograms of a commerial conjugated linoleic acid (CLA) preparation containing no CLA. The corresponding CLA regions in each chromatogram are labelled: x is an unknown component. The absorbance scale is shown in the lower graph to indicate the low response found in the CLA region.

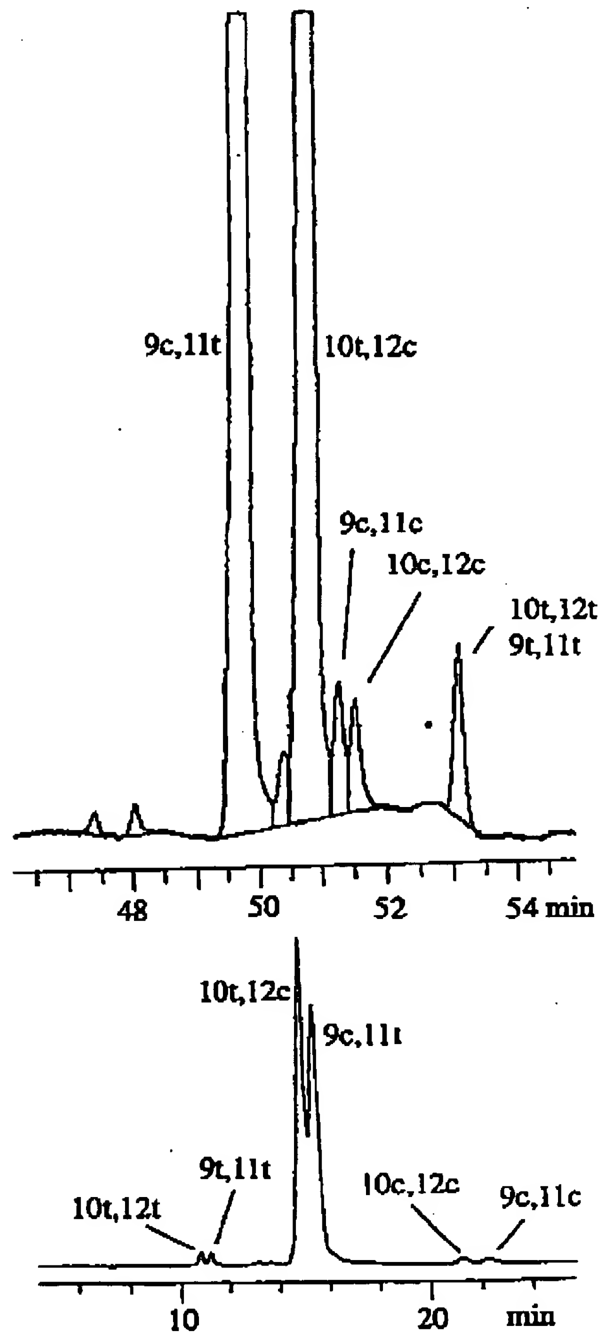

Fig. 2. Gas (upper graph) and silver ion-high performance liquid (lower graph) chromatograms of a representative commercial conjugated linoleic acid (CLA) preparation consisting primarily of two CLA isomers, 9 *cis*, 11 *trans*-18:2 and 10 *trans*, 12 *cis*-18:2. Peaks corresponding to CLA isomers in each chromatogram are labelled.

major fatty acids, other than the CLA isomers found in the preparations, included palmitic (16:0), stearic (18:0), oleic (18:1n-9), and linoleic (18:2n-6) acids. Their combined content ranged from 1 to 84% in the products examined. Based on the GC-internal standard technique, total CLA content ranged from 0 to 94% of the total FAMEs, or 0 to 89% of the mass content of the products.

The CLA isomer distributions in the products, analyzed by a combination of GC and Ag$^+$-HPLC, are shown in Tab. 1. No CLA was found in product 1. The CLA-containing products fell into two catagories: those composed of two major CLA positional isomers (9 *cis*, 11 *trans*-18:2 and 10 *trans*, 12 *cis*-18:2), and those composed of four major CLA positional isomers (8 *trans*, 10 *cis*-18:2; 9 *cis*, 11 *trans*-18:2; 10 *trans*, 12 *cis*-18:2, and 11 *cis*, 13 *trans*-18:2). Other minor CLA isomers were present at much lower concentrations, but are not reported. All the CLA products in capsule form contained the mixture of four isomers, while the liquid products contained either two or four CLA positional isomers. Representativ GC and Ag$^+$-HPLC chromatograms for these groups are shown in Figs. 1, 2, and 3, respectively.

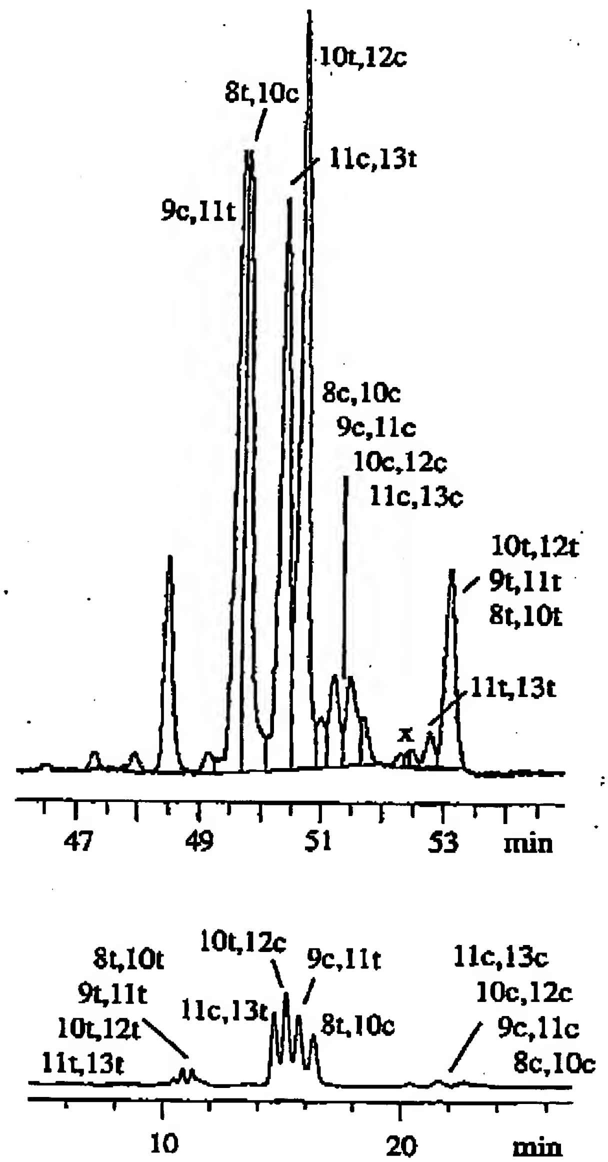

Fig. 3. Gas (upper graph) and silver ion-high performance liquid (lower graph) chromatograms of a representative commerial conjugated linoleic acid (CLA) preparation consisting primarily of four CLA isomers, 8 *trans*, 10 *cis*-18:2, 9 *cis*, 11 *trans*-18:2, 10 *trans*, 12 *cis*-18:2 and 11 *cis*, 13 *trans*-18:2. Peaks corresponding to CLA isomers in each chromatogram are labelled; x is an unknown component.

An explanation for the differences in isomer distributions among the products was not available. Alkali isomerization of 18:2 n-6 in laboratory scale batches prepared according to published procedures [12, 26, 27] resulted in the formation of only two CLA isomers, i.e., 9 *cis*, 11 *trans*-18:2 and 10 *trans*, 12 *cis*-18:2. Alkali isomerization under large-scale, and possibly more severe conditions, may have produced the four CLA positional isomer pattern observed in the commercial CLA preparations described here. This will need to be confirmed.

The GC analyses were based on use of a 100 m polar capillary column. In this system, 8 *trans*, 10 *cis*-18:2 was not resolved from 9 *cis*, 11 *trans*-18:2. A shoulder or a split peak may occasionally be evident when the amounts of these two isomers are approximately equal. In contrast, 11 *cis*, 13 *trans*-18:2 eluted before and was resolved from 10 *trans*, 12 *cis*-18:2 using this GC column (Fig. 3, upper graph). The *cis,cis* CLA isomers eluted after 10 *trans*, 12 *cis*-18:2 in the order 8, 10-, 9, 11-, 10, 12-, and 11,13-18:2 as established previously [10, 20]. The last CLA isomers to elute were the *trans,trans*, consisting of a small peak due to 11,13-18:2 followed by an unresolved mixture of 10,12-, 9,11-, and 8,10-18:2 as demonstrated previously [14, 17]. A small unknown peak was observed between the *cis,cis* and the *trans,trans* CLA isomer regions. The structural identity of all CLA isomers was established and confirmed by analyzing the DMOX derivatives of selective CLA products by GC-EIMS and GC-DD-FTIR. Representative mass and infrared spectra were published previously [10, 14, 17, 27].

Chromatograms showing the separation of the CLA isomers by Ag$^+$-HPLC are presented below the GC chromatograms in Figs. 1 to 3. The elution orders of all the geometric (in the order *trans,trans*, *cis/trans*, and *cis,cis*) and positional (in the order 11,13-, 10,12-, 9,11-, and 8,10-18:2) CLA isomers by Ag$^+$-HPLC were established previously [10]. Ag$^+$-HPLC was essential to complement the GC analysis and establish the composition of 8 *trans*, 10 *cis*-18:2 and 9 *cis*, 11 *trans*-18:2, and the distribution of most of the *trans,trans* CLA isomers.

In contrast to the commercial CLA preparations, that were found to contain two or four CLA positional isomers, natural dairy products and meats from ruminant animals contain primarily rumenic acid, 9 *cis*, 11 *trans*-18:2 [11, 12, 14, 17–19]. While it has not been established, which isomer(s) is (are) responsible for the reported beneficial properties of CLA, it is generally thought that anticarcinogenicity is due to rumenic acid [12, 15]. The nutritional and physiological effects, if any, of other CLA isomer(s) in commercially available CLA preparations are not known.

We recently reported, that one of the four major *cis/trans* CLA isomers, 11 *cis*, 13 *trans*-18:2, accumulates preferentially in heart phospholipids and specifically in heart and liver diphosphatidylglycerol (DPG) of pigs fed a CLA mixture containing four positional isomers [20]. DPG is a major component of inner mitochondrial membranes and is involved in many enzymes of bioenergetics in the mitochondria [28, 29]. *Watkins* et al. [30] demonstrated that docosahexaenoic acid (22:6 n-3) accumulated in DPG of human colonic adrenocarcinoma (HT-29) cells and increased mito-

chondrial oxidant production. Similarly, 11 *cis*, 13 *trans*-18:2 (or any other CLA isomer incorporated into DPG), could affect mitochondrial oxidant production, particularly since it has been shown that the xidativ susceptibility of CLA is comparable to that of arachidonic acid (20:4 n-6) [31, 32]. In response to our findings that 11 *cis*, 13 *trans*-18:2 was selectively incorporated into DPG [20], a major supplier of commercial CLA preparations recently modified the process to eliminate the 11 *cis*, 13 *trans*-18:2 isomer. The simultaneous elimination of 8 *trans*, 10 *cis*-18:2 from the resulting CLA mixture was an additional benefit of preparing a CLA mixture devoid of 11 *cis*, 13 *trans*-18:2.

In conclusion, the CLA products analyzed in this study were found to contain up to 12 geometric and positional CLA isomers. These findings are based on appropriate and improved analytical methodologies that have only recently been developed [10, 14, 16, 17, 20, 27]. All commercially available CLA products investigated differ, some significantly, and the isomers present may not necessarily represent active CLA components. As new products consisting of two or perhaps only one CLA isomer become available, it will be possible to determine the physiological effects of specific isomers. This is essential for an understanding of this unusual group of lipids.

Abbreviations

Ag^+-HPLC, high-performance liquid chromatography; *cis/trans*, refers to all the CLA isomers having either a *cis,trans* or a *trans,cis* configuration; CLA, conjugated linoleic acid; DMOX, 4,4-dimethyloxazoline; FAME, fatty acid methyl esters; GC-DD-FTIR, gas chromatography-direct deposition-Fourier transform infrared; GC-EIMS, gas chromatography-electron ionization mass spectrometry.

Acknowledgement

The authors wish to thank Dr. *Jeanne L Rader*, Office of Food Labeling, CFSAN, FDA, for her helpful suggestions and critical comments. Contribution number S019 from the Southern Crop Protection, Food Research Center, Agriculture and Agri-Food Canada, Guelph, ON, Canada.

References

[1] *C. Ip, C. Jiang, H.J. Thompson*, and *J.A. Scimeca*, Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis, Carcinogenesis 18 (1997), 755–759.

[2] *P. Knekt, R. Järvinen, R. Seppänen, E. Pukkala*, and *A. Aromaa*, Intake of dairy products and the risk of breast cancer, Br. J. Cancer 73 (1996), 687–691.

[3] *K.N. Lee, D. Kritchevsky*, and *M.W. Pariza*, Conjugated linoleic acid and atherosclerosis in rabbits, Atherosclerosis 108 (1994), 19–25.

[4] *R.J. Nicolosi, E.J. Rogers, D. Kritchevsky, J.A.Scimeca*, and *P.J. Huth*, Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters, Artery 22 (1997), 266–277.

[5] *K.L. Houseknecht, J.P. Vanden Heuvel, S.Y. Moya-Camarena, C.P. Portocarrero, L.W. Peck, K.P. Nickel*, and *M.A. Belury*, Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat, Biochem. Biophys. Res. Comm. 244 (1998), 678–682.

[6] *S.F. Chin, J.M. Storkson, K.J. Albright, M.E. Cook*, and *M.W. Pariza*, Conjugated linoeic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency, J. Nutr. 124 (1994), 2344–2349.

[7] *Y. Park, K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook*, and *M.W. Pariza*, Effect of conjugated linoleic acid on body composition in mice, Lipids 32 (1997), 853–858.

[8] *M.E.R. Dugan, J.L. Aalhus, A.L. Schaefer*, and *J.K.G. Kramer*, The effects of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs, Can. J. Anim. Sci. 77 (1997), 723–725.

[9] *Y. Li* and *B.A. Watkins*, Conjugated linoleic acids alter bone fatty acid composition and reduce *ex vivo* prostaglandin E_2 biosynthesis in rats fed n-6 or n-3 fatty acids, Lipids 33 (1998), 417–425.

[10] *N. Sehat, M.P. Yurawecz, J.A.G. Roach, M.M. Mossoba, J.K.G. Kramer*, and *Y. Ku*, Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers, Lipids 33 (1998), 217–221.

[11] *P.W. Parodi*, Conjugated octadecadienoic acids of milk fat, J. Dairy Sci. 60 (1977), 1550–1553.

[12] *S.F. Chin, W. Liu, J.M. Storkson, Y.L. Ha*, and *M.W. Pariza*, Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens, J. Food Comp. Anal. 5 (1992), 185–197.

[13] *J.K.G. Kramer, P.W. Parodi, R.G. Jensen, M.M. Mossoba, M.P. Yurawecz*, and *R.O. Adlof*, Rumenic acid: a proposed common name for the major conjugated linoleic acid isomer found in natural products, Lipids 33 (1998), 835.

[14] *M.P. Yurawecz, J.A.G. Roach, N. Sehat, M.M. Mossoba, J.K.G. Kramer, J. Fritsche, H. Steinhart*, and *Y. Ku*, A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis*-octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue, Lipids 33 (1998), 803–809.

[15] *Y.L. Ha, N.K. Grimm*, and *M.W. Pariza*, Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses, J. Agric. Food Chem. 37 (1989), 75–81.

[16] *F. Lavillonnière, J.C. Martin, P. Bougnoux*, and *J.-L. Sébédio*, Analysis of conjugated linoleic acid isomers and content in French cheeses, J. Am. Oil Chem. Soc. 75 (1998), 343–352.

[17] *N. Sehat, J.K.G. Kramer, M.M. Mossoba, M.P. Yurawecz, J.A.G. Roach, K. Eulitz, K.M. Morehouse*, and *Y. Ku*, Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences, Lipids 33 (1988), 963–971.

[18] *A.C. Fagerty, G.L. Ford*, and *D. Svoronos*, Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk, Nutr. Rep. Internat. 38 (1988), 937–944.

[19] *H. Lin, T.D. Boylston, M.J. Chang, L.O. Luedecke*, and *T.D. Shultz*, Survey of the conjugated linoleic acid contents of dairy products, J. Dairy Sci. 78 (1995), 2358–2365.

[20] *J.K.G. Kramer, N. Sehat, M.E.R. Dugan, M.M. Mossoba, M.P. Yurawecz, J.A.G. Roach, K. Eulitz, J.L. Aalhus, A.L. Schaefer*, and *Y. Ku*, Distribution of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high performance liquid chromatography, Lipids 33 (1998), 549–558.

[21] *N. Hashimoto, T. Aoyama*, and *T. Shioiri*, New methods and reagents in organic synthesis. 14. A simple efficient preparation of methyl esters with trimethylsilyldiazomethane ($TMSCHN_2$) and its application to gas chromatographic analysis of fatty acids, Chem. Pharm. Bull. 29 (1981), 1475–1478.

[22] *M.P. Yurawecz, J.K. Hood, J.A.G. Roach, M.M. Mossoba, D.H. Daniels, Y. Ku, M.W. Pariza*, and *S.F. Chin*, Conversion of allylic hydroxy oleate to conjugated linoleic acid and methoxy oleate by acid-catalyzed methylation procedures, J. Am. Oil Chem. Soc. 71 (1994), 1149–1155.

[23] *M.M. Mossoba, R.E. McDonald, D.J. Armstrong*, and *S.W. Page*, Identification of minor C_{18} triene and conjugated diene isomers in hydrogenated soybean oil and margarine by GC-MI-FT-IR spectroscopy, J. Chromatogr. Sci. 29 (1991), 324–330.

[24] *M.M. Mossoba*, Applications of capillary GC-FTIR, Inform 4 (1993), 854–859.

[25] *J.K.G. Kramer, V. Fellner, M.E.R. Dugan, F.D. Sauer, M.M. Mossoba*, and *M.P. Yurawecz*, Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids, Lipids 32 (1997), 1219–1228.

[26] *C.R. Scholfield* and *S. Koritala*, A simple method for preparation of methyl *trans*-10, *cis*-12-octadecadienoate, J. Am. Oil Chem. Soc. 47 (1970), 303.

[27] *N. Sehat, R. Rickert, M.M. Mossoba, J.K.G. Kramer, M.P. Yurawecz, J.A.G. Roach, R.O. Adlof, K.M. Morehouse, J. Fritsche, H. Steinhart,* and *Y. Ku,* Improved separation of conjugated fatty acid methyl esters by silver ion-high performance liquid chromatography, Lipids, in press.

[28] *F.L. Hoch,* Cardiolipins and Biomembrane Function, Biochim. Biophys. Acta **1113** (1992), 71–133.

[29] *M.K. Shigenaga, T.M. Hagen,* and *B.N. Ames,* Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. USA **91** (1994), 10771–10778.

[30] *S.M. Watkins, L.C. Carter,* and *J.B. German,* Docosahexaenoic acid accumulates in cardiolipin and enhances HT-29 cell oxidant production, J. Lipid Res. **39** (1998), 1583–1588.

[31] *J.J.M. Van den Berg, N.E. Cook,* and *D.L. Tribble,* Reinvestigation of the antioxidant properties of conjugated linoleic acid, Lipids **30** (1995), 599–605.

[32] *A. Zhang* and *Z.Y. Chen,* Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids, J. Am. Oil Chem. Soc. **74** (1997), 1611–1613.

Addresses of the authors: *Martin P. Yurawecz* (author to whom correspondence should be sent), *Najibullah Sehat, Magdi M. Mossoba, John A. G. Roach,* and *Youh Ku,* US Food and Drug Administration, Center for Food Safety and Applied Nutrition, 200 C St., S.W., Washington, DC 20204, USA; *John K. G. Kramer,* Southern Crop Protection, Food Research Center, Agriculture and Agri-Food Canada, Guelph, ON, N1G 2W1, Canada.

[Received: January 1, 1999; accepted: April 27, 1999].